MICRO BIOTEC'11

Microevolutionary changes of commercial *Saccharomyces cerevisiae* strains recovered from vineyard environments identified by comparative genome hybridization on array

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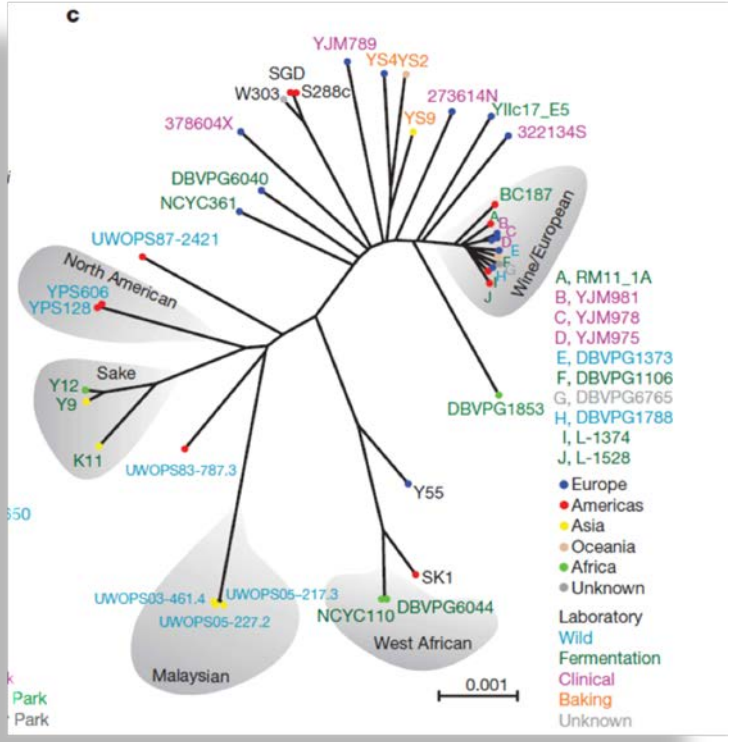
CBMA

CESAM

INRA
Institut National de la Recherche Agronomique

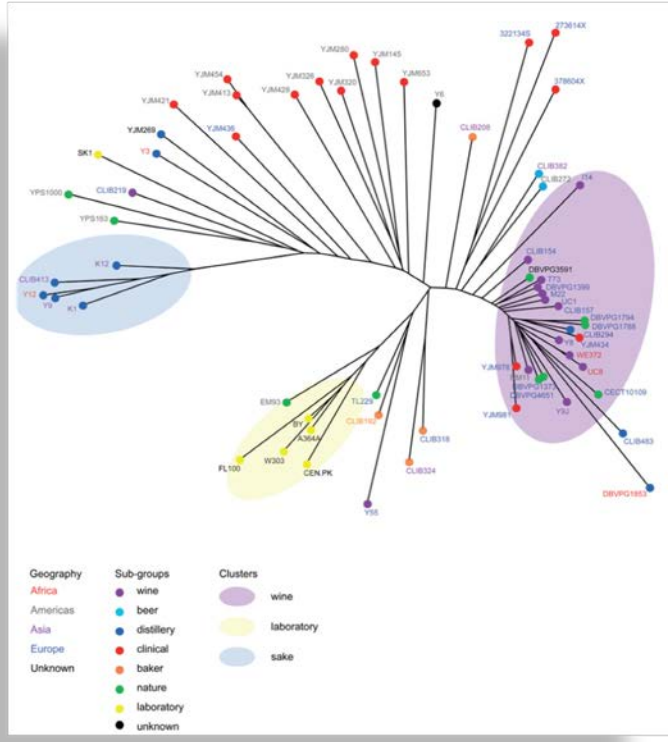
INTRODUCTION

The population structure of *Saccharomyces cerevisiae*



Liti et al., Nature, 2009

235,127 SNPs
14,051 nucleotide insertions or deletions



Schacherer et al., Nature, 2009

1.89 x 10^6 SNP (30,097 SNPs per strain)
3,985 deletios (200 bp length)

- low coverage genome sequencing
- high density arrays

- few well-defined, geographically isolated lineages
- many different mosaics of these lineages (wine, laboratory and saké strains)

INTRODUCTION

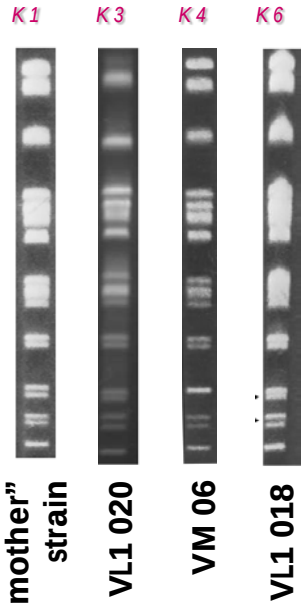
S. cerevisiae commercial winemaking strains

- ✓ Extensive use of commercial *S. cerevisiae* wine strains
- ✓ Such strains are disseminated from the winery and can be recovered from locations in close proximity (10-200m)

  Valero *et al.*, 2005
- ✓ Re-isolation of 100 isolates of the commercial strain VL1 from vineyards close to the winery where this strain has been used during many years

  Schuller and Casal, 2007

Karyotype patterns

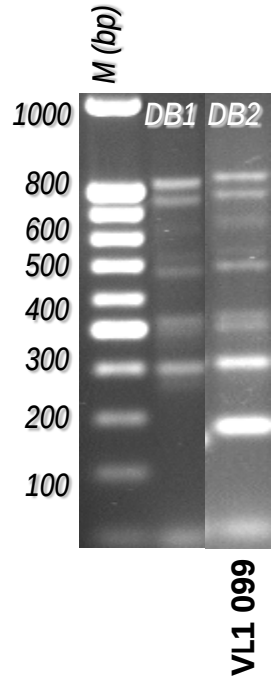


Microsatellite patterns

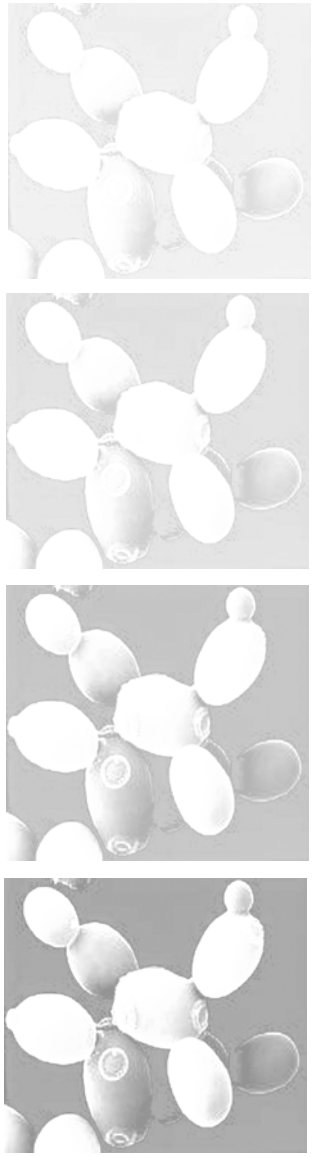
Loci	Alleles (bp) of distinct microsatellite patterns	
	M1	M7
ScAAT1	204/219	204/219
ScAAT2	372/381	372/381
ScAAT3	265	265
ScAAT4	329	329
ScAAT5	219/222	222
ScAAT6	256/259	256/259

"mother" Strain — VI1 020

Interdelta sequence amplification patterns



Objectives

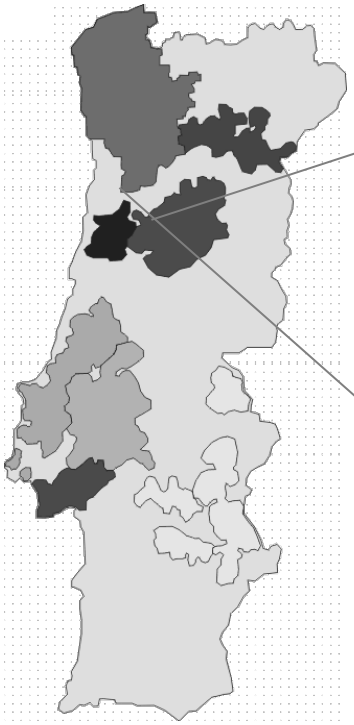


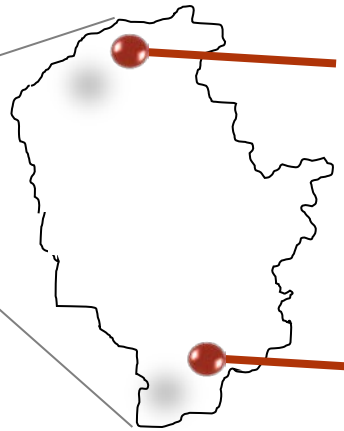
- ✓ Evaluation of genome variations among isogenic isolates of the commercial strain *Saccharomyces cerevisiae* Zymaflore VL1 that were re-isolated from vineyards surrounding the wineries where this industrial strain was applied, using Comparative Genome Hybridization on array (aCGH);

- ✓ Conclude about adaptive mechanisms that occur during the strain's permanence in vineyard environments

Materials and Methods

Saccharomyces cerevisiae isolates





VL1 099 and VL1 108

VL1 isolates recovered from vineyards

VL1 018 and VL1 020

Reference

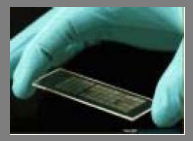
1. **Commercial VL1 "mother" strain**
2. **VM06** (Isolate obtained through clonal expansion of the "mother" strain)

Materials and Methods

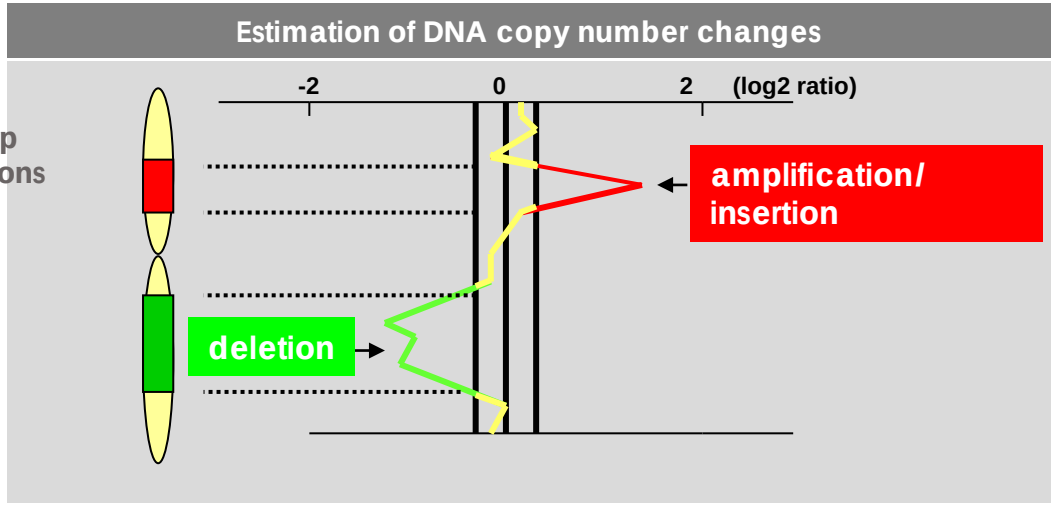
Array Chromosome Genome Hybridization (aCGH)

Reference DNA
"VL1 Mother strain"
Cy5

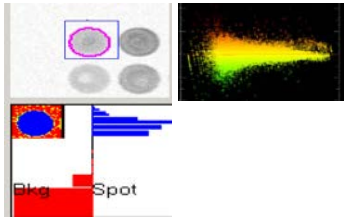
Test DNA
VM 06
VL 018
VL 020
VL 099
VL 108
Cy3



Dye swap hybridizations



QuantArray software Image analysis - data extraction

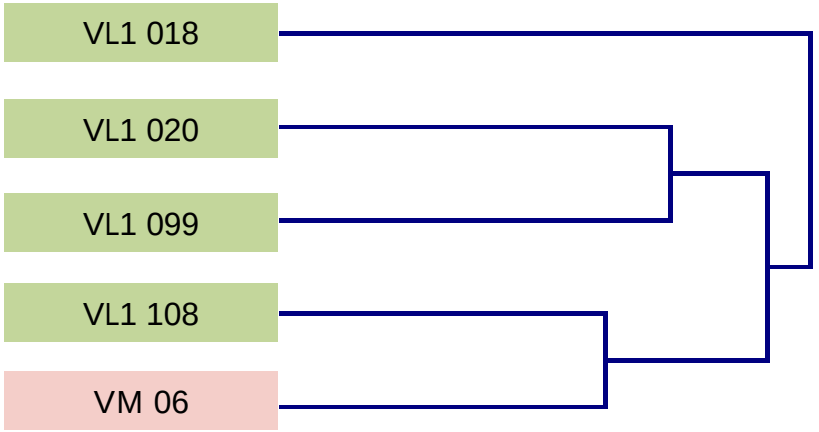


BrB software Normalization of data

MeV software Graphical displays of log ratios and visual representation of data
Significance Analysis for Microarrays

Results

Clustering of aCGH profiles

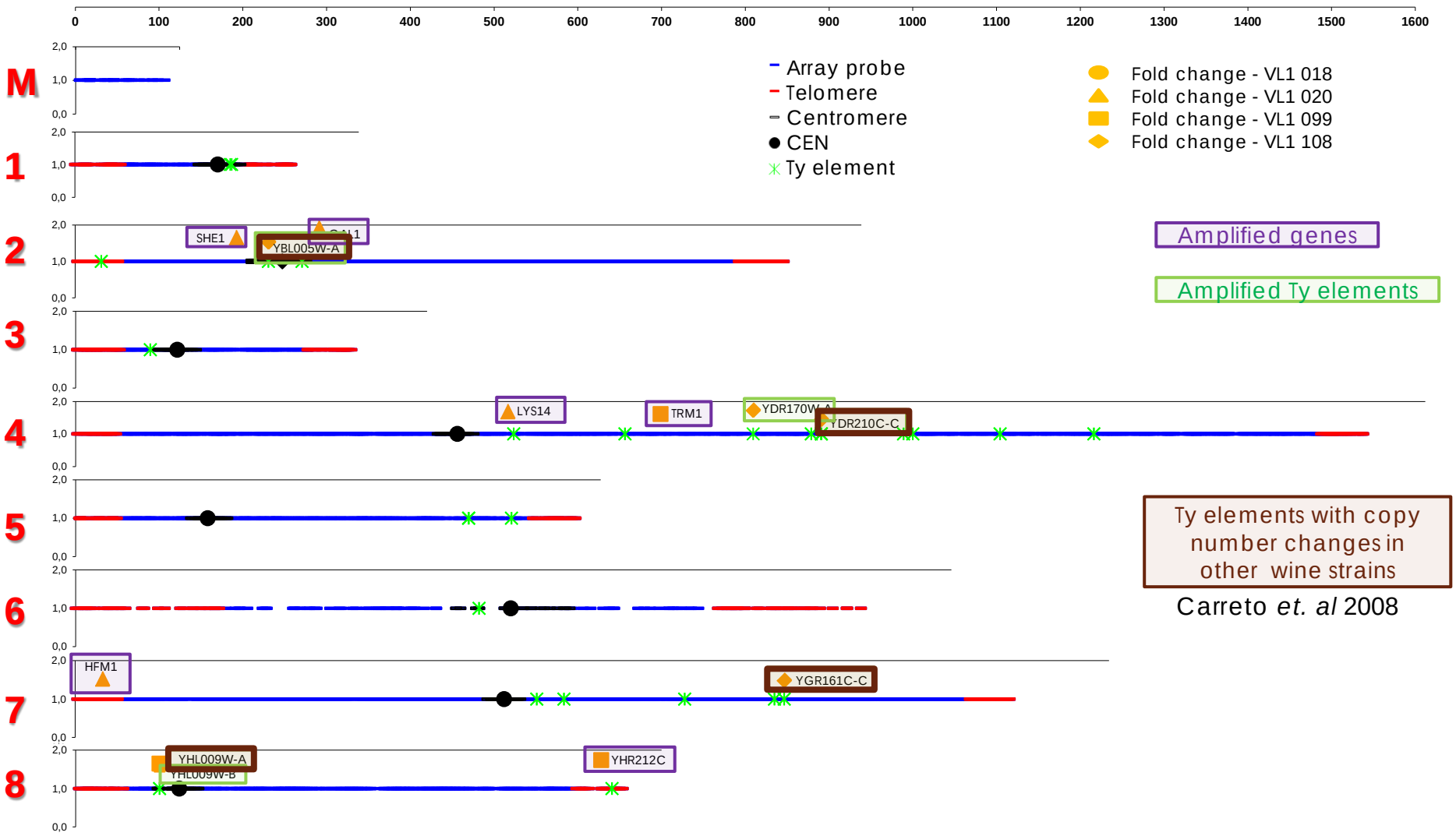


(Hierarchical clustering, Pearson correlation, average linkage)

No clear separation between VL1 isolates obtained from nature (●) and an isolate derived from the "mother" strain (●)

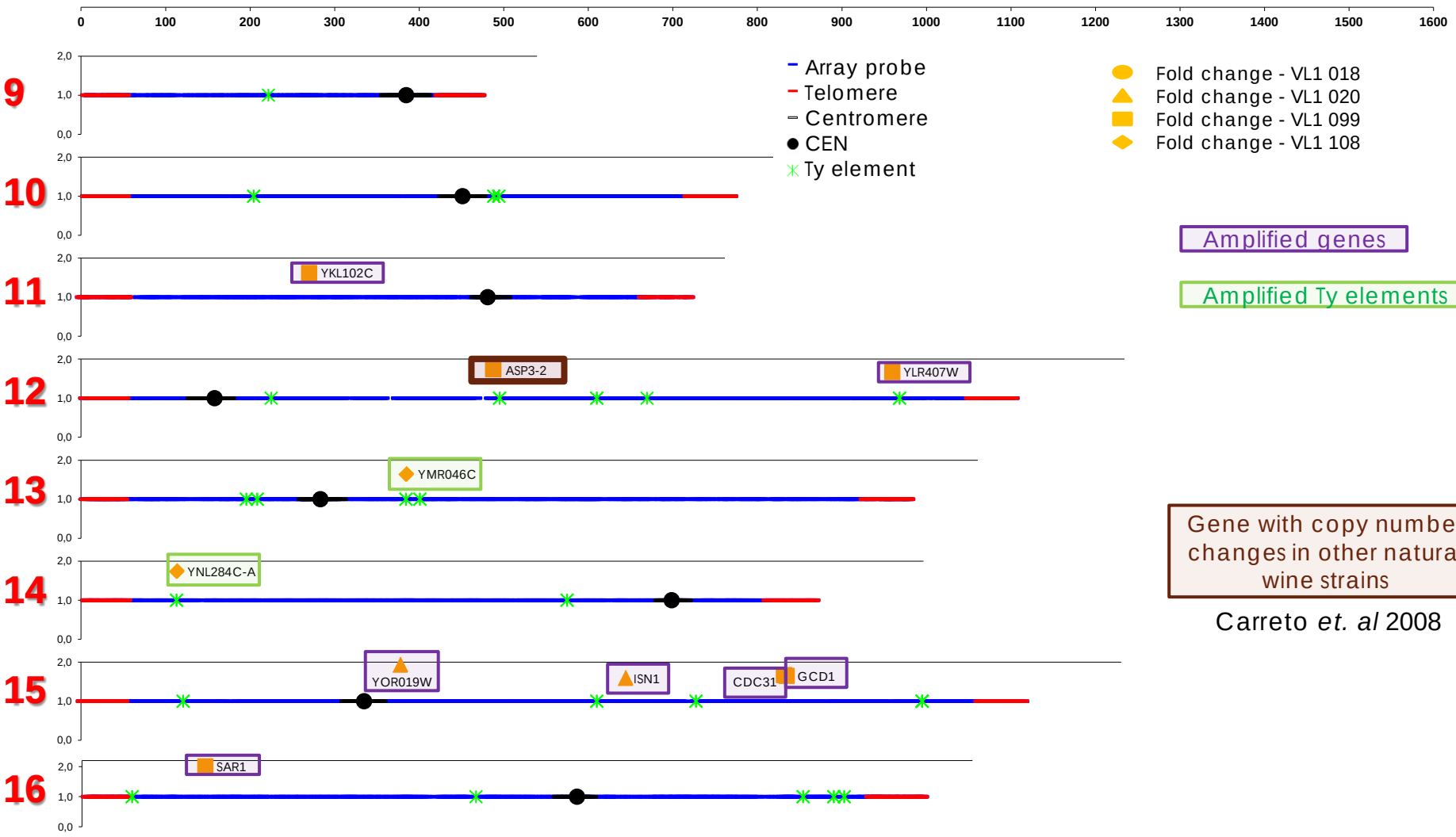
Results

Gene Copy number alterations – SAM analysis



Results

Gene Copy number alterations – SAM analysis



Amplified genes

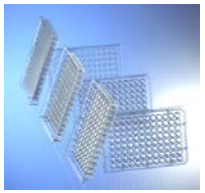
Amplified Ty elements

Gene with copy number changes in other natural wine strains

Carreto *et. al* 2008

Results

Phenotypic characterization

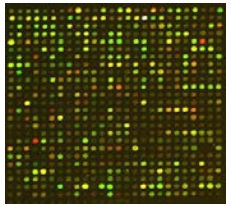


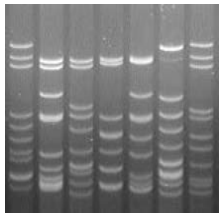
- Wine must + compound
- 30 ºC
- 200 rpm
- quadruplicate

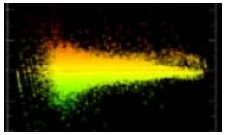
Strain	Phenotypic tests																		
	30°C	18°C	40°C	pH 2	pH 8	KCl 0.75M	NaCl 1.5M	CuSO4 5mM	SDS 0.01%	Etanol 6%	Etanol 10%	Etanol 14%	Iprodion (0.05mg/mL)	Iprodion (0.1mg/mL)	Procymidon (0.05mg/mL)	Procymidon (0.1mg/mL)	KHSO3 (150 mg/l)	$KHSO_3$ (300 mg/l)	Vinho + glucose 0.5%	Vinho + glucose 1%
VL1 018	3	1	3	0	2	2	1	0	0	3	2	1	3	3	3	3	3	1	1	1
VL1 020	3	1	3	0	2	3	1	0	0	3	2	1	3	3	3	3	3	1	1	1
VL1 099	3	1	3	0	2	2	1	0	0	3	2	1	3	3	3	3	3	2	0	0
VL1 108	3	1	3	0	2	2	0	0	0	3	2	1	3	3	3	3	3	2	0	0
VM06	3	1	3	0	2	2	1	0	0	3	2	1	3	3	3	3	3	2	1	1
"Mother" strain	3	0	3	0	2	2	1	1	1	3	2	1	3	3	3	3	3	2	0	1

0 – Abs_{640nm} 0.1
1 – Abs_{640nm} 0.2-0.4
2 – Abs_{640nm} 0.5-1.2
3 – Abs_{640nm} ≥1.3

SUMMARY AND CONCLUSIONS







- Isogenic isolates of the commercial wine yeast strain *Zymaflore VL1* recovered from nature show genetic differences in comparison with the "mother" strain:
  - Gene amplifications
  - *Ty* element amplifications
  - Apparent stochastic distribution
- Generation of intra-strain phenotypic variability

The transition from nutrient-rich musts to nutritionally scarce natural environments is correlated with microevolutionary changes that may reflect adaptative responses

Acknowledgements

- Dorit Schuller
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Innoyeast

SEVENTH FRAMEWORK PROGRAMME

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MINISTÉRIO DA CIÊNCIA, TECNOLOGIA E ENSINO SUPERIOR

FCT Fundação para a Ciência e a Tecnologia
MINISTÉRIO DA CIÊNCIA, TECNOLOGIA E ENSINO SUPERIOR

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